

# A new species of *Temnosewellia* (Platyhelminthes, Temnocephalida) ectosymbiont on *Villopotamon thaii* (Crustacea, Decapoda, Potamidae) from Vietnam

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## ABSTRACT

A new species of the genus *Temnosewellia* (Platyhelminthes, Temnocephalida, Temnocephalidae), *Temnosewellia vietnamensis* n. sp., is described from Quangnam Province, Vietnam, ectosymbiont on the external carapace of *Villopotamon thaii* Dang & Ho, 2003 (Crustacea, Decapoda, Potamidae). Records of temnocephalans from the region of Southeast Asia are superficial despite a great number of potential host species. *Temnosewellia semperi* (Weber, 1889), is the only known species and has been recorded from Aru, Java, Sumatra, Philippines, Malaysia, Thailand and China. In 2003, temnocephalans were reported for the first time from Vietnam which led to the current description of a new species based largely on characters from adults and eggs. The new species has five anterior tentacles; black pigment confined to the eyes; an ejaculatory sac; a cirrus straight that is not dilated and which has an obvious distal unspined region; anterior parenchyma with only two Haswell cells; yellow, slightly reniform eggs, that lack a peduncle and which are deposited on host carapace. The opercular plates of the eggs form a ring of seven almost rectangular shapes. The fracture plane of the operculum is parallel to the long axis of the egg. The great number of potential host species available in the Southeast Asia is taken as evidence that the region may harbour many other, yet undescribed, temnocephalan species.

## KEY WORDS

Platyhelminthes,  
"Turbellaria",  
Temnocephalidae,  
*Temnosewellia*,  
Southeast Asia,  
Vietnam,  
new species.

## RÉSUMÉ

Une nouvelle espèce de *Temnosewellia* (Platyhelminthes, Temnocephalida), ectosymbionte sur *Villopotamon thaili* (Crustacea, Decapoda, Potamidae) au Vietnam.

Une nouvelle espèce de *Temnosewellia* (Platyhelminthes, Temnocephalida, Temnocephalidae), *Temnosewellia vietnamensis* n. sp., est décrite. Elle est commensale de la surface extérieure de la carapace de *Villopotamon thaili* Dang & Ho, 2003 (Crustacea, Decapoda, Potamidae), récolté dans la province de Quangnam, au Vietnam. Les mentions de temnocéphales du Sud-Est asiatique sont rares, malgré le grand nombre d'espèces-hôtes potentielles, pouvant les héberger. *Temnosewellia semperi* (Weber, 1889) est la seule espèce connue, elle a été mentionnée de plusieurs localités dans la région (Aru, Java, Sumatra, Philippines, Malaisie, Thaïlande et Chine). La présence de temnocéphales au Vietnam a été mentionnée pour la première fois en 2003 et cela a amené à cette description d'une nouvelle espèce, basée principalement sur des caractères des adultes et des œufs. La nouvelle espèce possède une combinaison unique de caractères. Elle possède cinq tentacules antérieurs; du pigment noir confiné aux yeux; un sac éjaculateur; un cirrus droit, non dilaté, avec une région distale évidente dépourvue d'épines; un parenchyme antérieur avec deux cellules de Haswell; des œufs jaunes, sans pédoncule, légèrement reniformes, déposés sur la carapace de l'hôte. Les plaques operculaires des œufs forment un anneau de sept plaques presque rectangulaires et le plan de fracture de l'opercule est parallèle par rapport à l'axe majeur de l'œuf. La présence de cette nouvelle espèce de temnocéphale, avec sa combinaison spéciale de caractères, ainsi que le grand nombre d'espèces pouvant servir comme hôtes, suggèrent que la région du Sud-Est asiatique doit héberger de nombreuses autres espèces non encore décrites.

## MOTS CLÉS

Platyhelminthes,  
«Turbellaria»,  
Temnocephalidae,  
*Temnosewellia*,  
Asie du Sud-Est,  
Vietnam,  
espèce nouvelle.

## INTRODUCTION

Temnocephalans (Platyhelminthes, "Turbellaria") are rhabdocoels, ectosymbionts on freshwater crustaceans, molluscs, insects and chelonians; they are considered closely related to dalyellioids. Joffe & Cannon (1998) characterized the temnocephalans by a multisyncytial epidermis, divided into a series of plates, and a tendency to lose locomotor cilia, with a posterior adhesive organ. This small group of turbellarians is known mainly from Australia and South America.

Within this group, the family Temnocephalidae Monticelli, 1899 is the most diverse, with Gondwanan affinities. The Australian region is the best studied and shows the highest specific richness but the host diversity is low. Contrarily, South America

has a lower number of temnocephalid species, but a great diversity of hosts taxa.

The genus *Temnosewellia* Damborenea & Cannon, 2001 includes about 50 species (Cannon 1991; Cannon & Sewell 2001; Damborenea & Cannon 2001; Sewell *et al.* 2006), characterized by five anterior tentacles, posterior adhesive disc, without papillae on the dorsal surface or tentacular surface, with dark pigment and with five syncytial plates: tentacular, posttentacular, body, peduncular and adhesive plates; the excretory pore lies on the body plate. *Temnosewellia* is distributed throughout the Australian region and in addition it ranges across the oriental area, from Southern China to central India and Malaysia (Gelder 1999). *Temnosewellia* species are ectosymbionts of freshwater crayfish, especially of *Euastacus* Clark, 1936, *Cherax* Erichson,

1846 (Parastacidae Huxley, 1879), other Decapoda (Parathelphusidae Alcock, 1910, Potamidae Ortmann, 1896, Atyidae De Haan, 1849 and Palaeomonidae Rafinesque, 1815) and Isopoda.

References of southeastern Asian temnocephalids are superficial. *Temnosewellia semperi* (Weber, 1889) was mentioned from several localities in Southeast Asia (Aru, Java, Sumatra, Philippines, Malaysia, Thailand and China), associated with *Cherax* (Gelder 1999; Sewell *et al.* 2006; Xu *et al.* 2006). However, Thai & Pham (2003) recorded for the first time the presence of temnocephalans in Vietnam. They examined freshwater crabs (Potamidae) and found several specimens and egg capsules fixed on the carapace of collection specimens. The adult and eggs of this temnocephalan species are studied here.

Here we describe a new species belonging to *Temnosewellia*, a commensal of *Villopotamon thaii* Dang & Ho, 2003, collected in Quangnam province, Vietnam.

## MATERIAL AND METHODS

The specimens were collected from the “Soui Cat” Stream at Bana Nature Reserve (Danang City, Vietnam) by Dr Thai Bai. He and Prof. Marc Laulier sent us fixed specimens and wholemounts to study.

Wholemounts were stained with carmine chloride and mounted in synthetic Canada balsam. Serial sections for histology were obtained from worms embedded in Paraplast, cut at 4 µm, stained with hematoxylin and eosin and mounted in synthetic Canada balsam.

Two specimens were dissected to extract the cirrus. One was mounted in polyvinyl-lactophenol for optic microscope (OM) study and the other was dehydrated, dried, and metalized for scanning electron microscope (SEM) study.

For SEM observation, whole individuals and egg capsules were dehydrated in hexamethyldisilazane (HMDS), sputter coated with gold and examined with a JEOL 6360 SEM.

Silver nitrate (SN) was applied to identify the syncytial plates, but due to the previous fixation of

the specimens only limited results were obtained.

Photomicrographs were taken with a Zeiss Axio-plan 2 microscope. Photomicrographs of the cirrus were taken using Nomarski interference contrast filters. Measurements were obtained with the aid of an OM, ranges and number of specimen's measurement are following (between parentheses) the mean.

Terminology applied to describe the reproductive structures follows Cannon (1993), Sewell & Cannon (1998) and Sewell *et al.* (2006). Only few authors give importance to egg morphology of temnocephalan species in their descriptions (e.g., Hickman 1967; Amato *et al.* 2006; Volonteiro 2007). Nevertheless, a detailed description of them would offer additional features with taxonomic relevance.

From current knowledge about egg morphology, we propose a standard terminology for their description.

The eggs may be fixed to the host in two ways. This fact determines two opposed morphologies: 1) the longitudinal axis of the eggs is perpendicular to the fixation surface; this determines that the eggs are pyriform. These eggs can have a stalk of different length that joins the eggs with the substrate (stalked eggs/unstalked eggs). The distal end of the eggs (opposite to the fixation surface) can have a filament. This structure has different length and its position is also variable; being apical, or subapical, or displaced to the side;

2) the major axis of the eggs is parallel to the fixation surface. The eggs are reniform in shape (Fig. 5) and adhere by one plane side (Fig. 5C). The filament is also located on the opposite side of the fixation surface, and it can be on the central zone or slightly eccentric (Fig. 5) or near to one of the extremes of the long axis (Hickman 1967).

The operculum is the part of the egg that is lost during the eclosion. The operculum is set up by a central plate and a series of opercular plates placed in a ring; which are variable in number, size and shape (Fig. 5C-F) (Volonteiro 2007). The fracture plane is determined by their arrangement in relation to the biggest axis of the egg, resulting perpendicular or oblique fracture planes.

Several authors (Hickman 1967; Ponce de León 1989; Cannon 1993; Amato *et al.* 2006 and references

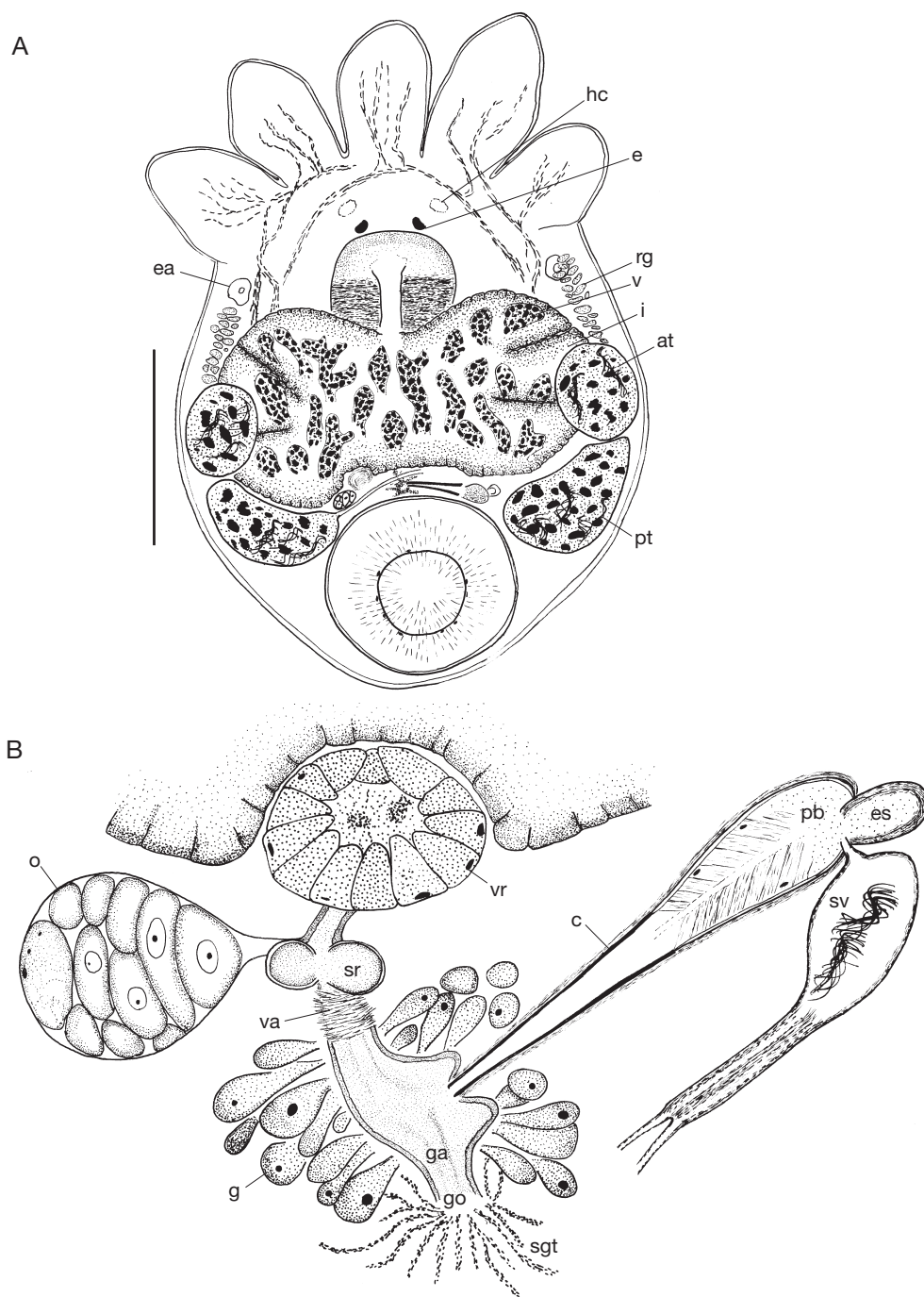


FIG. 1. — *Temnosewellia vietnamensis* n. sp.: **A**, drawing of a specimen in ventral view; **B**, reconstruction of the male and female genital systems. Abbreviations: **at**, anterior testicle; **c**, cirrus; **e**, eye; **ea**, excretory ampullae; **es**, ejaculatory sac; **g**, glands; **ga**, genital atrium; **go**, gonopore; **hc**, Haswell's cells; **i**, intestine; **o**, ovary; **pb**, prostatic bulb; **pt**, posterior testicle; **rg**, rhabdite glands; **sgt**, shell glands tracts; **sr**, seminal receptacle; **sv**, seminal vesicle; **v**, vitellaria; **va**, vagina; **vr** vesicula resorbens. Scale bars: A, 1000  $\mu$ m; B, 300  $\mu$ m.

therein) give eggs size (width and length). The filament and the peduncle length can be added.

Drawings were made with a drawing tube on Zeiss Standard 25 microscope.

Type and non-type material is deposited in the Muséum national d'Histoire naturelle, Paris (MNHN) and in the Invertebrate Collection of Museo de La Plata (MLP).

## SYSTEMATICS

Family TEMNOCEPHALIDAE Monticelli, 1899

Genus *Temnosewellia*

Damborenea & Cannon, 2001

*Temnosewellia vietnamensis* n. sp.  
(Figs 1-9)

TYPE MATERIAL. — Holotype: wholemount specimen (MNHN HEL 42). Paratypes: 2 specimens sagittally sectioned, 1 specimen frontally sectioned, 1 cirrus in polyvinyl-lactophenol (MLP 5744); 1 specimen transversally sectioned (MNHN HEL 43-HEL 50).

TYPE HOST. — *Villopotamon thaii* Dang & Ho, 2003.  
Site: external surface of the carapace.

TYPE LOCALITY. — Stream at Bana Nature Reserve (Quangnam province, Vietnam).

OTHER MATERIAL EXAMINED. — Same data as type material, 3 stained wholemount specimens, 3 wholemount specimens in polyvinyl-lactophenol, unhatched eggs, 3 SEM specimens and 1 cirrus mounted on stubs and coated with gold, and 32 specimens in 70% ethanol (MLP 5745, 5746, 5747); 8 wholemount specimens (MNHN HEL 42).

ETYMOLOGY. — The specific name refers to Vietnam, where the specimens were collected.

## DESCRIPTION

### *External characteristics*

Body about 2.5 mm (1.8-3.3 mm, 7) long without tentacles, and about 2.0 mm (1.5-2.6 mm, 7) wide; rounded to oval. Black pigment confined to eyes. Posterior adhesive disc pedunculate: disc diameter 809 µm at rim (624-1152 µm, 7), disc peduncle about 339 µm in diameter (240-456 µm, 7) (Figs 1A; 2; 3B). Epidermis thin, syncytial and unciliated.



FIG. 2. — *Temnosewellia vietnamensis* n. sp., photomicrographs of wholemount. Scale bar: 400 µm.

Through SEM and staining with SN the tentacular and peduncular syncytia were evident (Fig. 3C-E). The posterior border of the posttentacular plate was not evident.

### *Alimentary system*

Mouth mid-ventral, between the first and second quarters of body, surrounded by a muscular sphincter. Pharynx wider than long, 460 µm (340-700 µm, 6) long, 552 µm (440-650 µm, 6) wide, with a large sphincter; esophageal glands at its base. Intestine saccular, with septa (evident in the sections) in adults; intestinal walls very thick.

Paranephrocytes not evident.

### *Excretory system*

Excretory pores lateral to mouth, outside the posttentacular syncytial plates. Major excretory ducts inconspicuous.



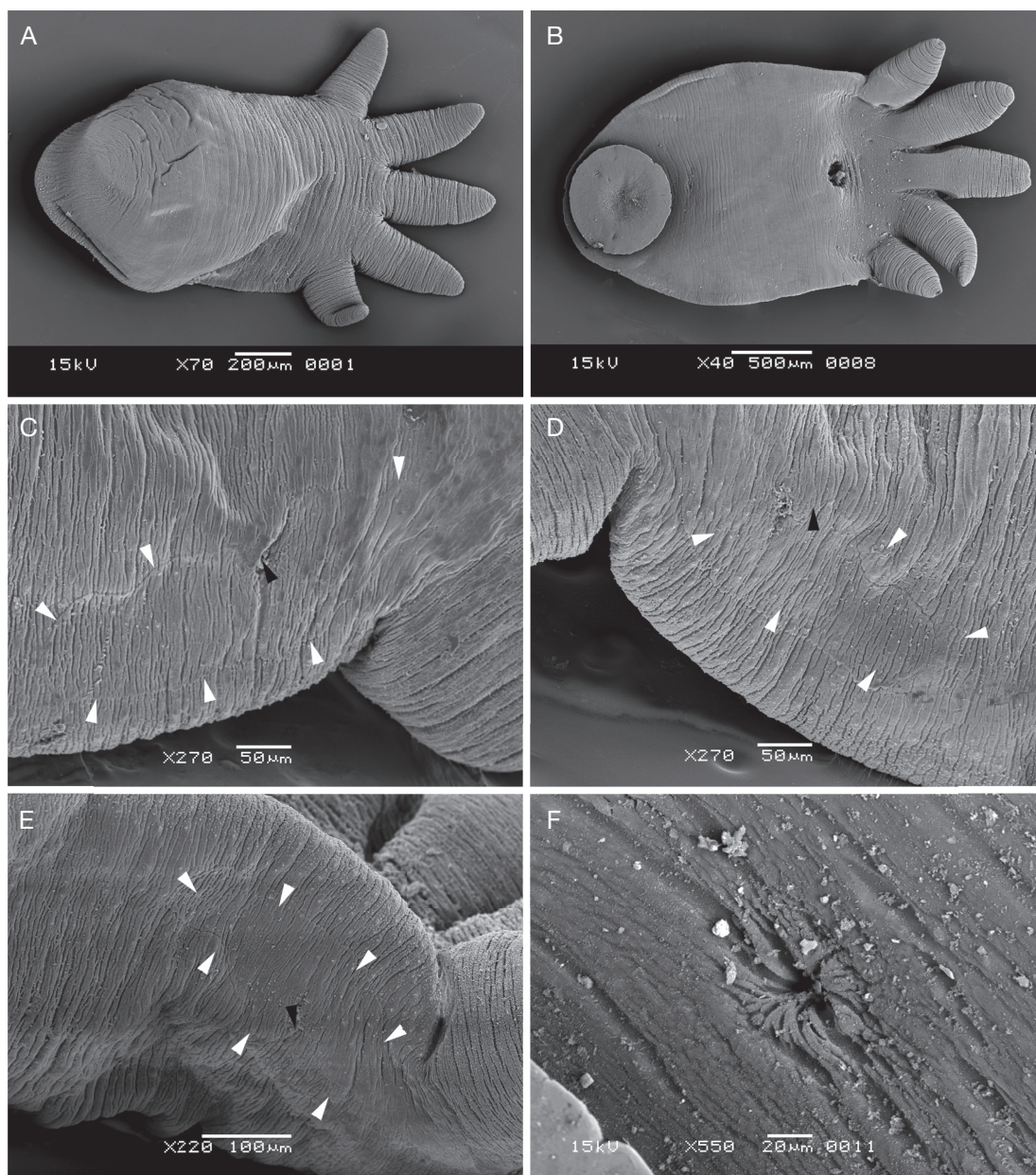


FIG. 3. — *Temnosewellia vietnamensis* n. sp.: **A, B**, SEM micrographs of whole specimens; **C–E**, details of the syncytial plates; **F**, detail of the gonopore. Black arrowheads show excretory pore, white arrowheads show the border of the postentacular and body syncytial plates. Scale bars: A, 200 µm; B, 500 µm; C, D, 50 µm; E, 100 µm; F, 20 µm.

### Glands

Rhabdite glands large, numerous, in lateral fields of the body, forming bunches, extending on the sides

of the intestinal sac, with conspicuous rhabdite tracts (Figs 1A; 2). Cyanophilus glands inconspicuous in wholmount specimens, evident in the sections,



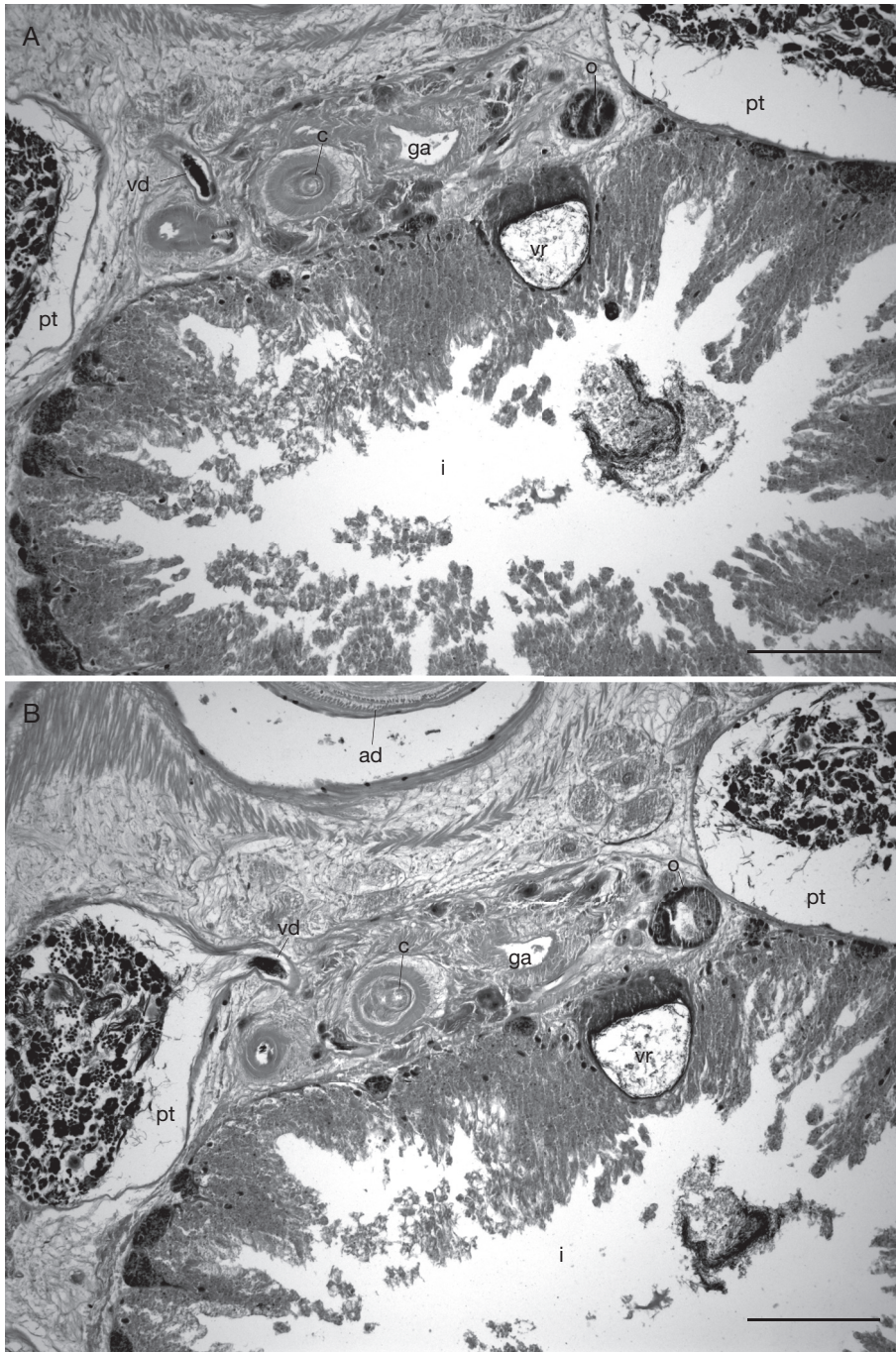


FIG. 4. — **A, B**, *Temnosewellia vietnamensis* n. sp., specimen frontally sectioned showing the arrangement and size of the genital structures, comprised between the posterior testicles, posterior wall of the intestine and the adhesive disc, and the conspicuous vesicula resorbens indenting the intestinal wall. Abbreviations: **ad**, adhesive disc; **c**, cirrus; **ga**, genital atrium; **i**, intestine; **o**, ovary; **pt**, posterior testicle; **vd**, vasa deferentia; **vr** vesicula resorbens. Scale bars: 200  $\mu$ m.

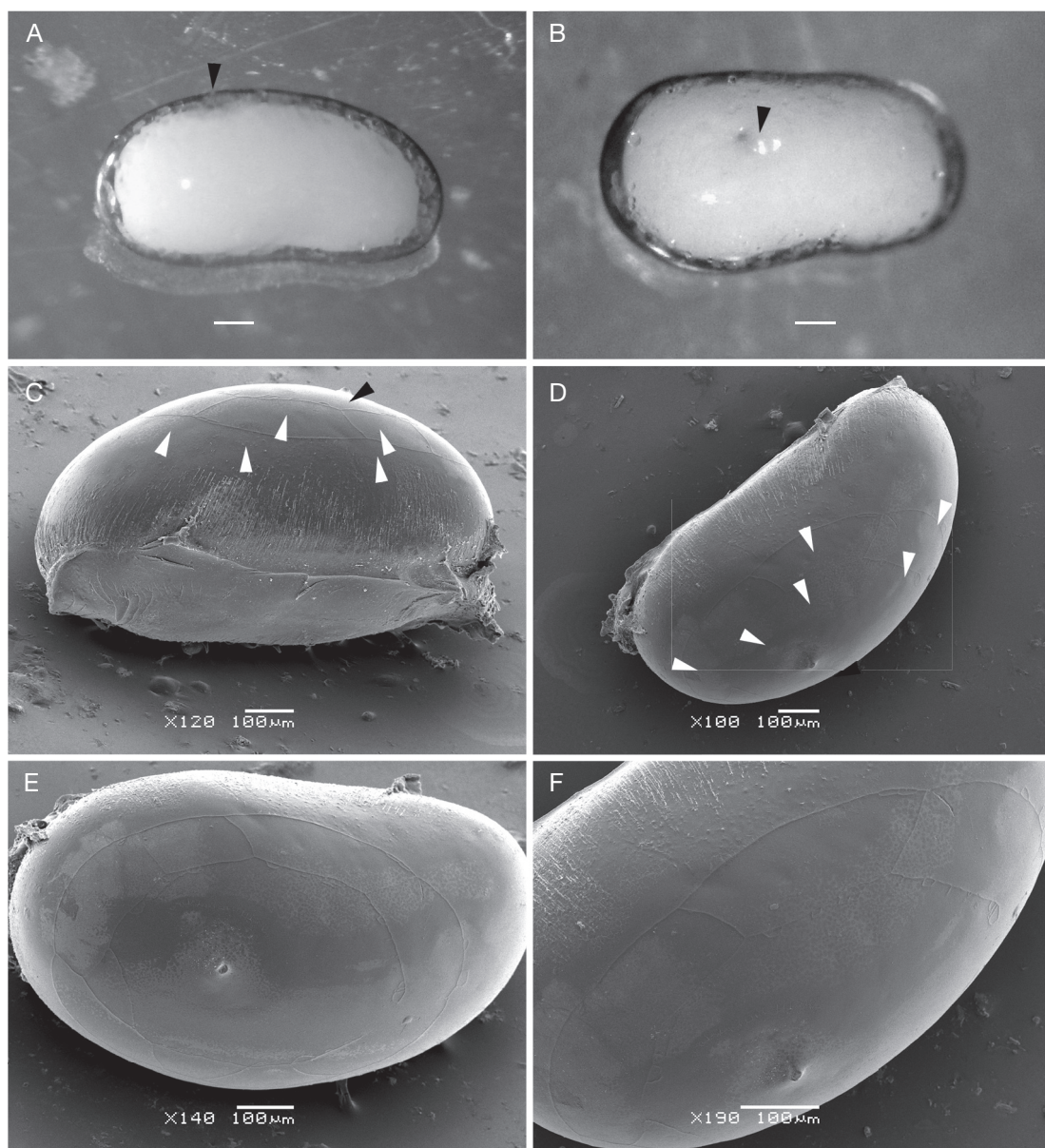


FIG. 5. — *Temnosewellia vietnamensis* n. sp., egg capsules: **A, B**, general views (binocular microscope); **C-F**, details of the opercular plates (SEM). White arrowheads show the opercular plates; black arrowheads show the filament. Scale bars: 100 μm.

separated in the parenchyma and located between the rhabdite glands. Two Haswell's cells in front of the eyes and the brain transverse band, showing little affinity with hematoxylin. Shell gland cells prominent, in lateral fields discharging to gonopore.

Disc glands present.

#### Muscles

Circular muscles of the body wall similar, dorsally and ventrally. Longitudinal muscles of body wall



stronger in the ventral surface. Attachment muscles of the adhesive disc and muscles controlling male organ very strong.

#### *Female reproductive system*

Gonopore mid-ventral, in the posterior third of the body (Fig. 3B, F); surrounded by a muscular sphincter. Genital atrium spacious, elongate. Ovary small (Figs 1B; 4); two seminal receptacles present, evident in sections; vitellaria covering dorsal and ventral sides of the intestinal sac, not extended outside its surface; vagina large and muscular specially in the proximal region, opening in front of the cirrus' introvert; vesicula resorbens elliptic, indenting intestinal sac and vitellaria posteriorly, from the left side, open to the gut in some specimens (Fig. 4).

Eggs yellow, without peduncle, deposited over the host carapace, 900  $\mu\text{m}$  long, 500  $\mu\text{m}$  wide, slightly reniform. The whole under surface of the egg capsule is cemented on the external surface of the host. Polar filament short (10  $\mu\text{m}$ ), on the upper surface, slightly eccentric (Fig. 5). Opercular plates form a ring of seven almost rectangular shapes. Fracture plane of the operculum parallel to the long axis of the egg.

#### *Male reproductive system*

Testes: anterior pair round and lateral to intestine sac; posterior pair always lateral and posterior to intestine, oblique, elliptical; always more voluminous than anterior testes, sometimes slightly lobed (Figs 1A; 2). Right anterior testis 436  $\mu\text{m}$  long (300–600, 6), 252  $\mu\text{m}$  (110–400, 6) wide; right posterior testis 518  $\mu\text{m}$  (240–80, 6) long, 342  $\mu\text{m}$  (150–450, 6) wide; left anterior testis 463  $\mu\text{m}$  (330–600, 6) long, 276  $\mu\text{m}$  wide (130–450, 6); left posterior testis 535  $\mu\text{m}$  (290–710, 6) long, 283  $\mu\text{m}$  (140–420, 6) wide. Vasa deferentia wide swollen, uniting separately to a large, pyriform, thick-walled, seminal vesicle. Ejaculatory sac present, with narrowed neck. Prostate bulb not well defined, as a continuation of the cirrus base (Fig. 1B). Cirrus straight (Fig. 6), 309.4  $\mu\text{m}$  long; 250  $\mu\text{m}$  shaft length; 73.78  $\mu\text{m}$  wide at proximal end; 11.9  $\mu\text{m}$  wide at distal end. Introvert 59.26  $\mu\text{m}$  long, not swollen, observed in different focusing planes (Fig. 7) with the Nomarski interference microscopy

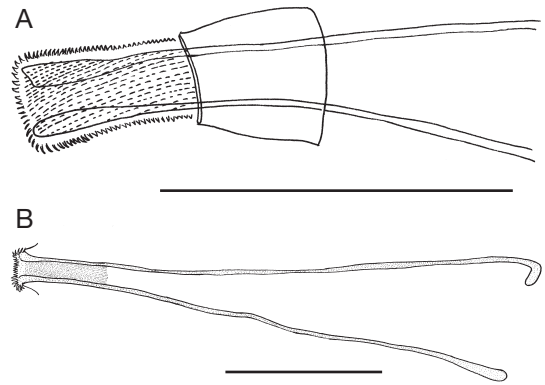


Fig. 6. — *Temnosewellia vietnamensis* n. sp., cirrus, detail of the distal extremity (A) and general view (B). Scale bars: 100  $\mu\text{m}$ .

(DIC). Proximal limit of introvert marked with a thickened ring, observed by SEM (Fig. 9). The eversible spined introvert has an evident unspined distal region (Figs 6; 7C–F; 8). The ratio between total length of cirrus and maximum width of shaft's proximal end 4.19; ratio between total length of cirrus and total length of introvert 5.22. Introvert spines very small, in approximately 40–45 parallel rows arranged slightly diagonal to the long axis of the introvert (Fig. 9A, D).

## DISCUSSION

Despite the great diversity of potential hosts within the family Potamidae from Southeast Asia, there are only a few records of Temnocephalidae species. *Temnosewellia vietnamensis* n. sp. is the first species of the family described for Vietnam. *Temnosewellia semperi* has been mentioned for several islands of the region (Aru, Celebes, Java and Sumatra). From mainland Southeast Asia this species is known from Malaysia, Thailand, China and India (Gelder 1999 and references therein; Xu *et al.* 2006). This species occurs in several crustaceans that belong in the family Potamidae.

Another species recorded in Southeast Asia is *Temnosewellia minor* (Haswell, 1887), in Japan, but it was introduced attached to the brown crayfish, introduced in a facility from Western Australia (Gelder 1999).

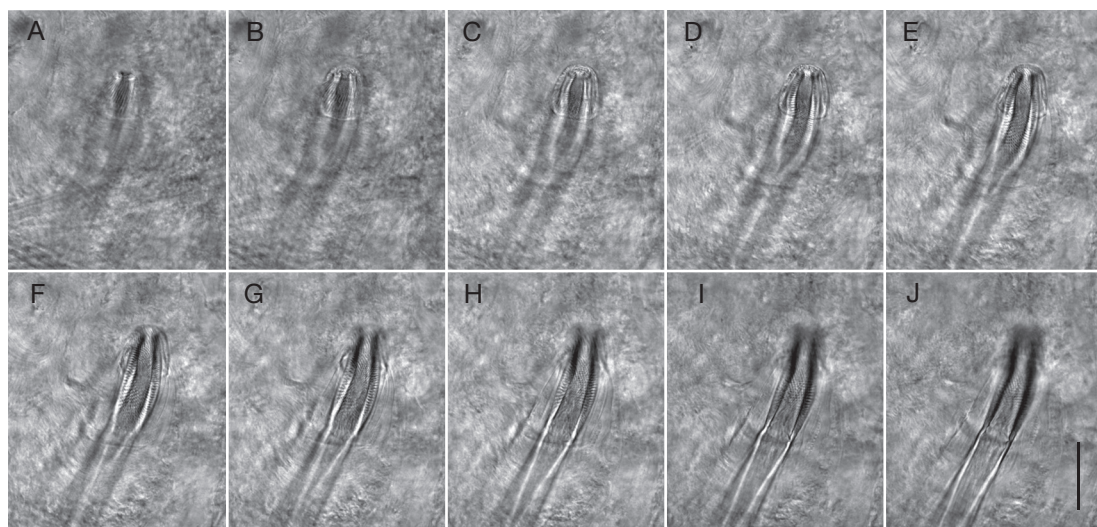


FIG. 7. — *Temnosewellia vietnamensis* n. sp., the cirrus introvert observed in different focusing planes with the Nomarski differential interference contrast (DIC) microscopy. Focus series captured from the top left hand corner to the bottom right hand corner. Scale bar: 25  $\mu$ m.

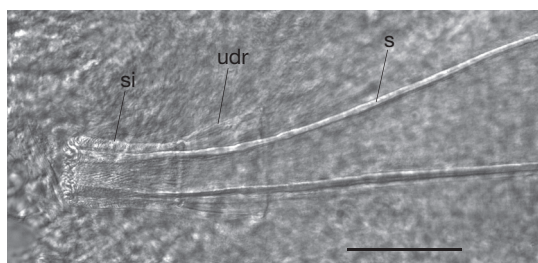


FIG. 8. — *Temnosewellia vietnamensis* n. sp., the cirrus introvert observed with the Nomarski differential interference contrast (DIC) microscopy showing the unspined distal region. Abbreviations: s, stylet; si, spined introvert; udr, unspined distal region. Scale bar: 50  $\mu$ m.

*Temnosewellia vietnamensis* n. sp. has the diagnostic features of the genus. Nevertheless, the egg capsules of *T. vietnamensis* n. sp. do not have a peduncle to cement to the substrate. On the contrary, they are firmly cemented by their under surface. This type of egg capsules is similar to that described for the species of *Temnomonticellia* Pereira & Cuocolo, 1941 (*T. tasmanica* (Haswell, 1900), *T. quadricornis* (Haswell, 1887), *T. aurantica* (Haswell, 1900), *T. pygmaea* (Hickman, 1967) and *T. fulva* (Hickman, 1967)), but the species of this

genus have four tentacles and a short central lobe (Hickman 1967).

*Temnosewellia vietnamensis* n. sp. has black pigment confined to the eyes. There are about 13 species that show this pigment distribution (Cannon 1993; Sewell *et al.* 2006). Among them, the species more closely similar to *T. vietnamensis* n. sp. are *Temnosewellia semperi*, from Indonesia to India, and *Temnosewellia rouxii* (Merton, 1914), from Aru Island (Merton 1914) and Australia (Cannon 1991). These two species are very similar among each other and also with *T. vietnamensis* n. sp.

Merton (1914) described the anatomy of *T. semperi* and *T. rouxii*. He did not compare in detail the features that are now considered to be of great systematic value (e.g., details of the cirrus morphology; female ducts structure; male glands). Only sporadic short accounts, but not complete descriptions, have been published subsequently (Cannon 1991). The feature considered of relevant systematic value now is the cirrus. *Temnosewellia semperi* has a nearly straight cirrus with the distal part slightly dilated. The cirrus of the new species is straight, not dilated and has an evident distal unspined region.

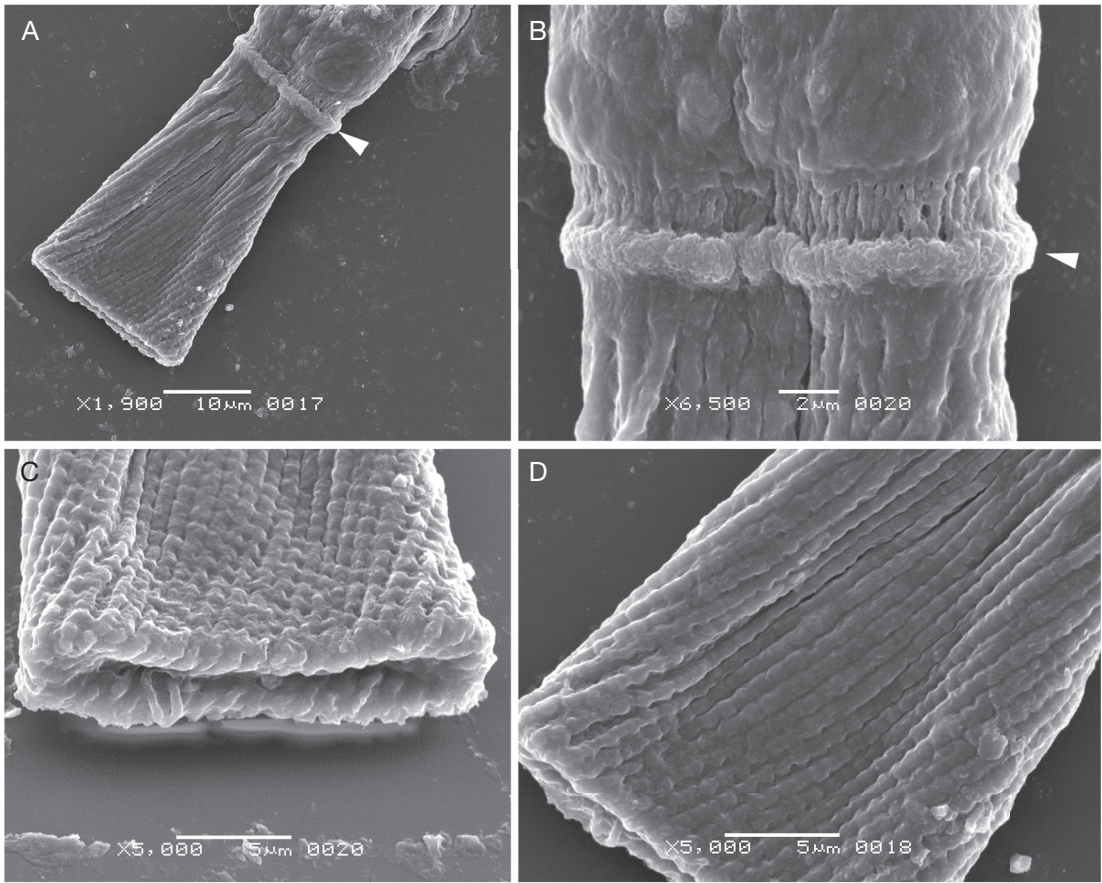


FIG. 9. — *Temnosewellia vietnamensis* n. sp., cirrus seen with SEM: **A**, detail of the introvert; **B**, detail of the thickened ring; **C**, frontal view of the introvert; **D**, detail of the rows of spines and their insertion points. White arrowheads show the thickened ring. Scale bars: A, 10 µm; B, 2 µm; C, D, 5 µm.

Nevertheless, the known species have a postentactular arc of large gland cells, anterior to the excretory ampullae (Merton 1914; Cannon 1991), not observed in the total and sectioned specimens of *T. vietnamensis* n. sp. The anterior parenchyma of the new species only has two cells, considered as Haswell's cells. No other glands have been observed.

The presence of this new temnocephalid species with its special combination of characters, and the great number of potential hosts species suggest that the Southeast Asian region might have many undescribed temnocephalid species. Cannon & Sewell (2001) suggest (after a personal communication of Joffe) that both *T. semperi* and *T. rouxii* may belong to a separate genus. This assertion needs to

be tested by detailed study of temnocephalans from this region designed to establish the relationship of the Australian and Asian species.

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